Design and Synthesis of Proteolysis Targeting Chimeras for Inducing BRD4 Protein Degradation

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Abstract In this paper, we synthesized a series of proteolysis targeting chimeras(PROTACs) using VHL E3 ligase ligands for BRD4 protein degradation. One of the most promising compound **19**g exhibited robust potency of BRD4 inhibition with IC_{50} value of (18.6±1.3) nmol/L, respectively. Furthermore, compound **19**g potently inhibited cell proliferation in BRD4-sensitive cell lines RS4;11 with IC_{50} value of (34.2±4.3) nmol/L and capable of inducing degradation of BRD4 protein at 0.4—0.6 μmol/L in the RS4;11 leukemia cells. These data show that compound **19**g is a highly potent and efficacious BRD4 degrader.

Keywords Proteolysis targeting chimera(PROTAC); BRD4 degrader; VHL ligand

1 Introduction

The proteolysis targeting chimeras(PROTACs) are heterobifunctional molecules consisting of a ligand that binds the protein of interest(POI) connected *via* a linker domain to a recruitment moiety for an E3 ubiquitin ligase. The proximity of the protein to the ligase can then induce the polyubiquitylation of the protein, and subsequent degradation *via* the proteasome[1]. The development of PROTACs as potential drugs capable of recruiting target proteins to the cellular machinery for elimination has created alternative options to tackle traditionally "difficult to drug" proteins[2]. A variety of protein types have been successfully degraded using this approach, including transcription factors, kinases and nuclear epigenetic readers using a variety of ligases, including Von Hippel-Lindau(VHL)[3], mouse double minute 2 homologue(MDM2)[4], F-box/WD repeat containing protein(*β*-TrCP)[5], cereblon[6,7], and inhibitor of apoptosis protein(IAP)[8].

With VHL, three different classes of VHL-targeting PROTACs were made to target the bromodomain-containing protein 4(BRD4), the receptor interacting serine/threonine protein kinase 2(RIPK2), and the nuclear hormone receptor estrogen-related receptor *α*(ERR*α*)[9,10]. BRD4 is an exciting new therapeutic target for cancer and other human diseases[11—13]. PROTACs MZ1[9] and ARV-771[14](Fig.1) employing BRD4 ligand JQ-1, which has successfully used VHL E3 ligase

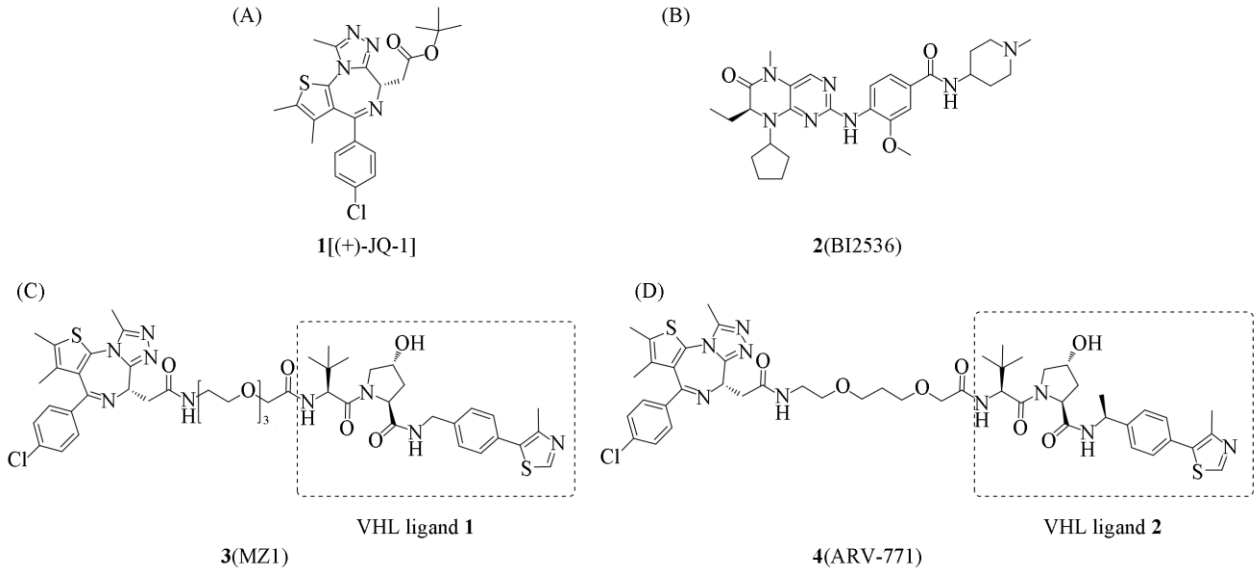

Fig.1 Chemical structures of the reported BRD4 inhibitors 1(A) and 2(B) and BRD4 degraders 3(C) and 4(D)

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ligands have been shown to efficiently induce BRD4 protein degradation and to be more potent in the inhibition of cancer cell growth and in the induction of apoptosis than their corresponding BRD4 inhibitors. BI2536(Fig.1) is a potent BRD4 inhibitor[15]. In this research, we reported the discovery of a new class of VHL E3 ligase-based BRD4 PROTACs designed based upon BI2536 and VHL ligands. Their biological activities were also evaluated.

2 Experimental

2.1 Materials and Instruments

All necessary chemical materials in the experiments were analytical grade. ^{1}H NMR spectra of the products were recorded on a Bruker 400 MHz spectrometer with tetramethylsilane(TMS) as an internal standard. HR-MS spectra were determined on an Agilent Q-TOF-6250 spectrometer.

2.2 Synthesis of the Target Compounds

*2.2.1 Synthesis of (R)-Methyl 2-(Cyclopentylamino)butanoate(**6**)*

Compound **5**(10 g, 85 mmol) and cyclopentanone(5.5 g, 66 mmol) were dissolved in 100 mL of DCM. Sodium acetate(5.5 g, 66 mmol) and sodium triacetoxyborohydride(20 g, 95 mmol) were then added at 0 °C. The reaction mixture was stirred for 16 h at room temperature and then 200 mL of 20% $NaHCO_3$ solution was added. The aqueous phase was extracted with DCM. The combined organic phases were washed with water, dried over $MgSO_4$ and the solvent was removed under reduced pressure, affording compound **6**(11 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 3.68(s, 3H), 3.17(t, J=6.6 Hz, 1H, CH), 2.96—2.92(m, 1H, CH), 1.83—1.54(m, 6H, CH_2), 1.46(dd, J_1=9.0 Hz, J_2=5.5 Hz, 2H, CH_2), 1.26—1.24(m, 2H, CH_2), 0.87(t, J=7.5 Hz, 3H, CH_3).

2.2.2 Synthesis of (R)-Methyl 2-[(2-Chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino]butanoate(7)

Compound **6**(5.7 g, 31 mmol), potassium carbonate(4.3 g, 31 mmol) and 2,4-dichloro-5-nitropyrimidin(6.5 g, 34 mmol) were suspended in 150 mL of acetone. The mixture was stirred at room temperature overnight and the volatiles were removed under reduced pressure. To the residue was added 100 mL of water and the mixture was extracted with ethyl acetate. The combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude was purified by chromatography using petroleum ether/ethyl acetate(volume ratio, 20:1) to give compound **7**(5.3 g, 50%) as a yellow pow- der. ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.65(s, 1H, pyrimidine-H), 3.81—3.63(m, 4H, CH_2 CH_3), 3.61—3.45(m, 1H, CH), 2.48—2.32(m, 1H, CH), 2.28—2.12(m, 1H, CH), 2.09—2.02(m, 2H, CH_2), 1.88—1.46(m, 6H, CH_2), 1.04(t, J=7.5 Hz, 3H).

*2.2.3 Synthesis of (R)-2-Chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one(**8**)*

Compound **7**(3.8 g, 11 mmol) was dissolved in 50 mL of glacial acetic acid and 1.5 g of iron powder was added at 70 °C. The mixture was stirred for 1 h at 70 °C, then for 1.5 h at 100 °C. The solution was then filtered. The solvent was evaporated under reduced pressure and purified by chromatography using petroleum ether/ethyl acetate(volume ratio, 20:1) to give Compound **8**(1.5 g, 50%) as a yellow powder. ^{1}H NMR(400 MHz, $CDCl_3$), δ: 10.03(s, 1H, NH), 7.72(s, 1H, pyrimidine-H), 4.38—4.24(m, 1H, CH), 4.20(dd, J_1=7.2 Hz, J_2=3.1 Hz, 1H, CH), 2.16—2.00(m, 1H, CH), 1.96—1.82(m, 5H, CH, CH_2), 1.78(dt, J_1=21.8 Hz, J_2=7.3 Hz, 1H, CH), 1.69—1.56(m, 2H, CH_2), 0.92(t, J=7.4 Hz, 3H, CH_3).

*2.2.4 Synthesis of (R)-Methyl 2-[(2-Chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino]butanoate(**9**)*

Compound **8**(1.5 g, 5.3 mmol) and methyl iodide(0.6 mL, 9.5 mmol) were dissolved in 20 mL of DMF. The reaction was cooled down to −10 °C and sodium hydride(60%, 0.4 g, 9.5 mmol) was added. The reaction mixture was stirred for 0.5 h at 0 ºC, then for 1 h at room temperature. The reaction was then quenched by adding crushed ice. The solvent was evaporated under reduced pressure and the crude compound was taken up into ethyl acetate and washed twice with water. The organic layer was dried using $MgSO_4$ and volatiles were removed under reduced pressure affording compound **9**(1.5 g, 96%) as a light yellow solid. ^{1}H NMR(400 MHz, $CDCl_3$), δ: 7.68(s, 1H, pyrimidine-H), 4.40—4.27(m, 1H, CH), 4.26(dd, J_1=7.4 Hz, J_2=3.4 Hz, 1H, CH), 3.32(s, 3H, CH_3), 2.13—2.00(m, 1H, CH), 1.98—1.54(m, 9H, CH_2, CH), 0.86(t, J=7.5 Hz, 3H, CH_3).

*2.2.5 Synthesis of (R)-4-[(8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)-amino]-3-methoxybenzoic Acid(**10**)*

Compound **9**(1.5 g, 5.1 mmol) and 4-amino-3-methoxybenzoic acid(1.0 g, 6.0 mmol) were suspended in a mixture of 3 mL of ethanol, 15 mL of water and 15 μL of concentrated hydrochloric acid and refluxed for 48 h. Solvent was evaporated under reduced pressure and purified by chromatography using methanol/dichloromethane(volume ratio, 1:10) to give compound **10**(1.5 g, 70%) as a yellow powder. ^{1}H NMR(400 MHz, $CDCl_3$), δ: 9.65(s, 1H, COOH), 7.94(d, J=8.1 Hz, 1H, ArH), 7.87(s, 1H, pyrimidine-H), 7.61(dd, J_1=8.1 Hz, J_2=1.4 Hz, 1H, ArH), 7.58(d, J=1.9 Hz, 1H, ArH), 4.49—4.44(m, 1H, CH), 4.19—4.12(m, 1H, CH), 3.92(s, 3H, CH_3), 3.54(s, 1H, CH), 3.22(s, 3H, CH_3), 2.03—1.66(m, 6H, CH_2), 1.58—1.36(m, 4H, CH_2), 0.75(t, J=7.4 Hz, 3H, CH_3).

2.2.6 Preparation of Compounds 12

$(Boc)_2O$(5.5 g, 25.0 mmol) was added to a mixture of compound **11**(20.0 mmol), $NaHCO_3$(1.7 g, 20.0 mmol) in 20 mL of water and 20 mL of ethyl acetate at 5 °C. The reaction continued for 2 h. The reaction mixture was filtered. The solid was collected and suspended in a mixture of 20 mL of hexane and 20 mL of water for 0.5 h. The mixture was filtered, and the solid was collected and dried to afford compound **12** as a white solid.

Tert-butyl 4-bromobenzylcarbamate(**12**a): yield 85%, ^{1}H NMR(400 MHz, CD_3OD), δ: 7.44(d, J=8.3 Hz, 2H, ArH), 7.15(d, J=8.3 Hz, 2H, ArH), 4.26(s, 2H, CH_2), 1.49(s, 9H, CH_3).

(*S*)-Tert-butyl[1-(4-bromophenyl)ethyl]carbamate(**12**b):

yield 90%, ^{1}H NMR(400 MHz, CD_3OD), δ: 7.49(d, J=8.4 Hz, 2H, ArH), 7.25(d, J=8.4 Hz, 2H, ArH), 4.60—4.55(m, 1H, CH), 1.36(s, 9H, CH_3), 1.28(d, J=7.2 Hz, 3H, CH_3).

2.2.7 Preparation of Compounds *13*

A mixture of compound **12**(15.0 mmol), 4-methylthiazole(3.0 g, 30.0 mmol), palladium(II) acetate(34 mg, 0.15 mmol) and potassium acetate(3.0 g, 30.0 mmol) in 20 mL of DMF was stirred at 90 °C under nitrogen for 24 h. After cooling to room temperature, the reaction mixture was filtered. 100 mL of H_2O was added to the filtrate and the resulting mixture was stirred for 4 h. The reaction mixture was filtered. The solid was collected by filtration and dried to afford a gray solid. This solid was dissolved in 4 mol/L HCl in methanol(20 mL), and the mixture was stirred at room temperature for 3 h. The mixture was filtered, and the solid was collected and dried to afford compound **13** as a light green solid.

[4-(4-Methylthiazol-5-yl)phenyl]methanamine hydrochloride(**13**a): yield 76%, ^{1}H NMR(400 MHz, CD_3OD), δ: 8.87(s, 1H, thiazole-H), 7.52—7.42(m, 4H, ArH), 3.85(s, 2H, CH_2), 2.47(s, 3H, CH_3).

(*S*)-1-[4-(4-Methylthiazol-5-yl)phenyl]ethanamine hydrochloride(**13**b): yield 68%, ^{1}H NMR(400 MHz, CD_3OD), δ: 9.17(s, 1H, thiazole-H), 7.67(d, J=8.4 Hz, 2H, ArH), 7.57(d, J=8.4 Hz, 2H, ArH), 4.47—4.41(m, 1H, ArH), 2.48(s, 3H, CH_3), 1.56(d, J=6.8 Hz, 3H, CH_3).

2.2.8 Synthesis of (2S,4R)-1-{(S)-2-[(Tert-butoxycarbonyl)amino]-3,3-dimethylbutanoyl}-4-hydroxypyrrolidine-2-carboxylic Acid(*16*)

HATU(2.15 g, 5.7 mmol) was added to a solution of compound **14**(1.25 g, 5.4 mmol), compound **15**(1.0 g, 5.4 mmol), and DIPEA(2.5 g, 19.0 mmol) in 10 mL of DMF at 0 °C under N_2. The mixture was stirred at room temperature for 24 h. To the reaction mixture was added 50 mL of water and the mixture was extracted with ethyl acetate. The combined organic layer was washed twice with 5% citric acid, twice with saturated $NaHCO_3$ solution, and twice with brine and was dried by Na_2SO_4. The organic solution was filtered and concentrated to afford as yellow oil. This crude product and LiOH(1.2 g, 50 mmol) were added to a mixture of 20 mL of THF and 10 mL of water. The mixture was stirred at room temperature overnight. THF was removed by concentration. The residue was diluted with 10 mL of ice water and the pH was slowly adjusted to 2—3 with 3 mol/L HCl. The resulting suspension was filtered and washed with water. The solid was collected by filtration and was dried to afford compound **16**(1.3 g, 70%) as a white solid. ^{1}H NMR(400 MHz, DMSO-d_6), δ: 6.50(brs, 1H, COOH), 5.19(brs, 1H, OH), 4.32(brs, 1H, NH), 4.25(t, J=8.4 Hz, 1H, CH), 4.16(d, J=9.2 Hz, 1H, CH), 3.66—3.57(m, 2H, CH_2), 2.13—2.08(m, 1H, CH), 1.91—1.85(m, 1H, CH), 1.38(s, 9H, CH_3), 0.94(s, 9H, CH_3).

2.2.9 Preparation of Compounds *17*

HATU(1.6 g, 4.2 mmol) was added to a stirred solution of compound **16**(3.5 mmol), compound **13**(3.5 mmol) and DIPEA(1.36 g, 10.5 mmol) in anhydrous THF(20 mL) at 0 °C. The resulting mixture was stirred at room temperature for 2 h. THF was removed by concentration. Water(20 mL) was added and the resulting mixture was stirred for 4 h and then was filtered. The solid was collected and dried at 50 °C to give a white solid. This solid was dissolved in 4 mol/L HCl in 20 mL of methanol and stirred at room temperature for 3 h. The reaction mixture was concentrated to remove all volatiles under reduced pressure to give a light yellow solid. The solid was added to 20 mL of ether and stirred at ambient temperature for 4 h. The reaction mixture was filtered and the solid was collected and dried to afford compound **17**.

(2*S*,4*R*)-1-[(*S*)-2-Amino-3,3-dimethylbutanoyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide hydrochloride(**17**a): yield 55%, ^{1}H NMR(400 MHz, CD_3OD), δ: 8.67(s, 1H, thiazole-H), 7.46—7.32(m, 4H, ArH), 4.78—4.74(m, 1H, CH), 4.68—4.64(m, 1H, CH), 4.48(s, 1H, CH), 4.30—4.26(m, 1H, CH), 4.18(d, J=8.8 Hz, 1 H, CH), 3.62—3.58(m, 2H, CH_2), 2.52(s, 3H, CH_3), 2.40—2.36(m, 1H, CH), 2.14—2.09(m, 1H, CH), 0.93(s, 9H, CH_3).

(2*S*,4*R*)-1-[(*S*)-2-Amino-3,3-dimethylbutanoyl]-4-hydroxy-*N*-{(*S*)-1-[4-(4-methylthiazol-5-yl)phenyl]ethyl}-pyrrolidine-2-carboxamide hydrochloride(**17**b): yield 50%, ^{1}H NMR(400 MHz, CD_3OD), δ: 8.69(s, 1H, thiazole-H), 7.47(d, J=8.4 Hz, 2H, ArH), 7.41(d, J=8.4 Hz, 2H, ArH), 4.95—4.89(m, 1H, CH), 4.56(t, J=8.4 Hz, 1H, CH), 3.90—3.88(m, 1H, CH), 3.79—3.75(m, 1H, CH), 3.52—3.48(m, 1H, CH), 2.49(s, 3H, CH_3), 2.14—2.09(m, 1H, CH), 1.79—1.72(m, 1H, CH), 1.38(d, J=7.2 Hz, 3H, CH_3), 1.03(s, 9H, CH_3).

2.2.10 Preparation of Compounds *18*

Compound **17**(2.0 mmol) was added to a solution of azide carboxyl linker(2.4 mmol) in 50 mL of DCM. HATU(1.9 g, 5.0 mmol) and DIPEA(1.75 mL, 10 mmol) were then added. After stirring for 4 h at room temperature, the reaction mixture was extracted with water. The organic phase was dried over Mg_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography using methanol/dichloromethane(volume ratio, 1:10) to give the product **18**.

(2*S*,4*R*)-1-[(*S*)-2-(8-Azidooctanamido)-3,3-dimethylbutanoyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide(**18**a): yield 70%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.68(s, 1H, thiazole-H), 7.46—7.32(m, 4H, ArH), 4.76—4.72(m, 1H, CH), 4.64—4.48(m, 4H, CH_2), 4.32—4.28(m, 1H, CH), 4.15(d, J=8.8 Hz, 1H, CH), 3.62—3.58(m, 2H, CH_2), 3.26(t, J=6.9 Hz, 2H, CH_2), 2.56(s, 3H, CH_3), 2.40—2.36(m, 1H, CH), 2.34(t, J=7.6 Hz, 2H, CH_2), 2.14—2.09(m, 1H, CH), 1.69—1.54(m, 4H, CH_2), 1.43—1.30(m, 6H, CH_2), 0.96(s, 9H, CH_3).

(2*S*,4*R*)-1-[(*S*)-2-(8-Azidooctanamido)-3,3-dimethylbutanoyl]-4-hydroxy-*N*-{(*S*)-1-[4-(4-methylthiazol-5-yl)phenyl]ethyl}pyrrolidine-2-carboxamide(**18**b): yield 68%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.66(s, 1H, thiazole-H), 7.48—7.42(m, 4H, ArH), 4.95—4.90(m, 1H, CH), 4.58(t, J=8.4 Hz, 1H, CH), 3.92—3.88(m, 1H, CH), 3.79—3.75(m, 1H, CH), 3.52—3.48(m, 1H, CH), 3.28(t, J=6.9 Hz, 2H, CH_2), 2.49(s, 3H, CH_3), 2.36(t, J=7.6 Hz, 2H, CH_2), 2.14—2.09(m, 1H, CH), 1.79—1.72(m, 1H, CH), 1.69—1.56(m, 4H, CH_2),

1.43—1.30(m, 9H, CH_3, CH_2), 1.02(s, 9H, CH_3).

(2*S*,4*R*)-1-[(*S*)-2-{2-[2-(2-Azidoethoxy)ethoxy]-acetamido}-3,3-dimethylbutanoyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide(**18**c): yield 60%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.68(s, 1H, thiazole-H), 7.46—7.38(m, 4H, ArH), 4.76—4.72(m, 1H, CH), 4.68—4.64(m, 1H, CH), 4.48(s, 1H, CH), 4.30—4.26(m, 1H, CH), 4.20(s, 2H, CH_2), 4.18(d, *J*=8.8 Hz, 1H, CH), 3.78—3.58(m, 8H, CH_2), 3.43(t, *J*=5.2 Hz, 2H, CH_2), 2.56(s, 3H, CH_3), 2.42—2.36(m, 1H, CH), 2.16—2.10(m, 1H, CH), 0.98(s, 9H, CH_3).

(2*S*,4*R*)-1-[(*S*)-14-Azido-2-(tert-butyl)-4-oxo-6,9,12-trioxa-3-azatetradecan-1-oyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl]-benzyl)pyrrolidine-2-carboxamide(**18**d): yield 70%, ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.67(s, 1H, thiazole-H), 7.42—7.33(m, 4H, ArH), 4.78—4.75(m, 1H, CH), 4.59—4.53(m, 2H, CH_2), 4.46(d, *J*=8.4 Hz, 1H, CH), 4.36—4.30(m, 1H, CH), 4.14—4.12(m, 1H, CH), 4.05—3.96(m, 2H, CH_2), 3.69—3.64(m, 10H, CH_2), 3.62—3.58(m, 1H, CH), 3.37(t, *J*=10.1 Hz, 2H, CH_2), 2.85(s, 1H, CH), 2.63—2.58(m, 1H, CH), 2.52(s, 3H, CH_3), 2.14—2.09(m, 1H, CH), 0.95(s, 9H, CH_3).

(2*S*,4*R*)-1-[(*S*)-17-Azido-2-(tert-butyl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-1-oyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl]benzyl)pyrrolidine-2-carboxamide(**18**e): yield 65%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.68(s, 1H, thiazole-H), 7.38—7.30(m, 4H, ArH), 4.76—4.73(m, 1H, CH), 4.58—4.52(m, 3H, CH_3), 4.35(dd, J_1=5.3 Hz, J_2=14.9 Hz, 1H, CH), 4.10—4.07(m, 1H, CH), 4.05—3.87(m, 2H, CH_2), 3.68—3.60(m, 15H, CH_2, CH_3), 3.42—3.39(m, 2H, CH_2), 2.96(d, *J*=3.0 Hz, 1H, CH), 2.58—2.52(m, 4H, CH_2), 2.16—2.10(m, 1H, CH), 0.96(s, 9H, CH_3).

(2*S*,4*R*)-1-[(*S*)-2-{2-[2-(2-Azidoethoxy)ethoxy]-acetamido}-3,3-dimethylbutanoyl]-4-hydroxy-*N*-{(*S*)-1-[4-(4-methylthiazol-5-yl)phenyl]ethyl}pyrrolidine-2-carboxamide (**18**f): yield 70%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.67(s, 1H, thiazole-H), 7.48—7.40(m, 4H, ArH), 4.95—4.89(m, 1H, CH), 4.56(t, *J*=8.4 Hz, 1H, CH), 4.20(s, 2H, CH_2), 3.90—3.88(m, 1H, CH), 3.78—3.64(m, 7H, CH_2, CH_3), 3.52—3.48(m, 1H, CH), 3.44(t, *J*=5.2 Hz, 2H, CH_2), 2.49(s, 3H, CH_3), 2.16—2.10(m, 1H, CH), 1.79—1.72(m, 1H, CH), 1.38(d, *J*=7.2 Hz, 3H, CH_3), 0.96(s, 9H, CH_3).

(2*S*,4*R*)-1-[(*S*)-14-Azido-2-(tert-butyl)-4-oxo-6,9,12-trioxa-3-azatetradecan-1-oyl]-4-hydroxy-*N*-{(*S*)-1-{4-(4-methylthiazol-5-yl)phenyl]ethyl}pyrrolidine-2-carboxamide (**18**g): yield 75%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.69(s, 1H, thiazole-H), 7.48—7.40(m, 4H, ArH), 4.96—4.91(m, 1H, CH), 4.56(t, *J*=8.4 Hz, 1H, CH), 4.05—4.00(m, 2H, CH_2), 3.93—3.90(m, 1H, CH), 3.79—3.65(m, 11H, CH_2, CH_3), 3.52—3.48(m, 1H, CH), 3.40(t, *J*=10.1 Hz, 2H, CH_2), 2.52(s, 3H, CH_3), 2.15—2.09(m, 1H, CH), 1.79—1.72(m, 1H, CH), 1.38(d, *J*=7.2 Hz, 3H, CH_3), 0.98(s, 9H, CH_3).

(2*S*,4*R*)-1-[(*S*)-17-Azido-2-(tert-butyl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-1-oyl]-4-hydroxy-*N*-{(*S*)-1-[4-(4-methylthiazol-5-yl)phenyl]ethyl}pyrrolidine-2-carboxamide (**18**h): yield 70%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.67(s, 1H, thiazole-H), 7.48—7.39(m, 4H, ArH), 4.95—4.89(m, 1H, CH), 4.58(t, *J*=8.4 Hz, 1H, CH), 4.05—3.97(m, 2H, CH_2), 3.90—3.88(m, 1H, CH), 3.79—3.60(m, 15H, CH_2, CH_3), 3.52—3.48(m, 1H, CH), 3.42—3.39(m, 2H, CH_2), 2.50(s, 3H, CH_3), 2.14—2.09(m, 1H, CH), 1.79—1.72(m, 1H, CH), 1.38(d, *J*=7.2 Hz, 3H, CH_3), 0.98(s, 9H, CH_3).

*2.2.11 Preparation of Compounds **19***

Compound **18**(1.0 mmol) was dissolved in 20 mL of methanol, then 0.1 g of Pd/C(10%) was added and the reaction mixture was stirred under an atmosphere of H_2 for 6 h at room temperature. The reaction mixture was filtered and the resulting solution was evaporated to obtain the desired amine. The resulting amine(1.0 mmol) and compound **10**(0.4 g, 1.0 mmol) were dissolved in 50 mL of DCM. HATU(0.6 g, 1.5 mmol) and DIPEA(1.6 mL, 9.0 mmol) were added. After stirring the reaction mixture at room temperature overnight, the solvent was removed. The crude was purified by flash column chromatography using methanol/dichloromethane(volume ratio: 1:10) to give the product **19**.

(2S,4*R*)-1-{(*S*)-2-[8-(4-{[(*S*)-8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzamido)octanamido]-3,3-dimethylbutanoyl}-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide(**19**a): yield 50%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.67(s, 1H, thiazole-H), 7.94(d, *J*=8.1 Hz, 1H, ArH), 7.86(s, 1H, pyrimidine-H), 7.64—7.58(m, 2H, ArH), 7.46—7.32(m, 4H, ArH), 4.78—4.74(m, 1H, CH), 4.68—4.64(m, 1H, CH), 4.50—4.44(m, 2H, CH_2), 4.30—4.26(m, 1H, CH), 4.19—4.12(m, 2H, CH_2), 3.92(s, 3H, CH_3), 3.62—3.58(m, 3H, CH_3), 3.30—3.26(m, 2H, CH_2), 3.22(s, 3H, CH_3), 2.52(s, 3H, CH_3), 2.40—2.36(m, 1H, CH), 2.15—2.12(m, 1H, CH), 2.08—1.66(m, 8H, CH_2), 1.60—1.38(m, 8H, CH_2), 1.20—1.12(m, 6H, CH_3), 0.96(s, 9H, CH_3), 0.76(t, *J*=7.4 Hz, 3H, CH_3). HRMS, *m*/*z*, calcd. for $C_{52}H_{70}N_{10}O_7S([M+H]^+)$: 978.5150, found: 978.5154.

(2*S*,4*R*)-1-{(*S*)-2-[8-(4-{[(*S*)-8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzamido)octanamido]-3,3-dimethylbutanoyl}-4-hydroxy-*N*-{(*R*)-1-[4-(4-methylthiazol-5-yl)phenyl]ethyl}-pyrrolidine-2-carboxamide(**19**b): yield 52%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.69(s, 1H, thiazole-H), 7.96(d, *J*=8.1 Hz, 1H, ArH), 7.88(s, 1H, pyrimidine-H), 7.64—7.58(m, 2H, ArH), 7.47—7.41(m, 4H, ArH), 4.95—4.89(m, 1H, CH), 4.56(t, *J*=8.4 Hz, 1H, CH), 4.49—4.44(m, 1H, CH), 4.18—4.12(m, 1H, CH), 3.94(s, 3H, CH_3), 3.90—3.88(m, 1H, CH), 3.79—3.75(m, 1H, CH), 3.54—3.48(m, 2H, CH_2), 3.26—3.20(m, 5H, CH_2, CH), 2.54(s, 3H, CH_3), 2.16—2.09(m, 1H, CH), 2.04—1.66(m, 9H, CH_2, CH), 1.58—1.36(m, 11H, CH_3, CH_2), 1.18—1.13(m, 6H, CH_2), 1.02(s, 9H, CH_3), 0.75(t, *J*=7.4 Hz, 3H, CH_3). HRMS, *m*/*z*, calcd. for $C_{53}H_{72}N_{10}O_7S([M+H]^+)$: 992.5306, found: 992.5302.

(2*S*,4*R*)-1-[(*S*)-12-(Tert-butyl)-1-(4-{[(*S*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxyphenyl)-1,10-dioxo-5,8-dioxa-2,11-diazatridecan-13-oyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]-pyrrolidine-2-carboxamide(**19**c): yield 48%, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.69(s, 1H, thiazole-H), 7.98(d, *J*=8.1 Hz, 1H,

ArH), 7.86(s, 1H, pyrimidine-H), 7.64—7.56(m, 1H, ArH), 7.46—7.32(m, 4H, ArH), 4.80—4.76(m, 1H, CH), 4.70—4.66(m, 1H, CH), 4.52—4.44(m, 2H, CH_3), 4.30—4.26(m, 1H, CH), 4.18—4.12(m, 2H, CH_2), 4.02(s, 2H, CH_2), 3.92(s, 3H, CH_3), 3.70—3.58(m, 6H, CH_2), 3.56(t, *J*=6.0 Hz, 2H, CH_2), 3.54(s, 1H, CH), 3.40—3.34(m, 2H, CH_2), 3.22(s, 3H, CH_3), 2.54(s, 3H, CH_3), 2.40—2.36(m, 1H, CH), 2.16—2.12(m, 1H, CH), 2.03—1.66(m, 6H, CH_2), 1.58—1.36(m, 4H, CH_2), 0.98(s, 9H, CH_3), 0.76(t, *J*=7.4 Hz, 3H, CH_3). HRMS, *m*/*z*, calcd. for $C_{50}H_{66}N_{10}O_9S([M+H]^+)$: 982.4735, found: 982.4738.

(2*S*,4*R*)-1-[(*S*)-15-(Tert-butyl)-1-(4-{[(*S*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxyphenyl)-1,13-dioxo-5,8,11-trioxa-2,14-diazahexadecan-16-oyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide(**19**d): yield 50%, ^{1}H NMR(400 MHz, $CDCl_3$), *δ*: 8.86(s, 1H, thiazole-H), 7.96(d, *J*=8.1 Hz, 1H, ArH), 7.88(s, 1H, pyrimidine-H), 7.64—7.58(m, 2H, ArH), 7.38—7.33(m, 4H, ArH), 4.78—4.75(m, 1H, CH), 4.59—4.53(m, 2H, CH_2), 4.49—4.46(m, 2H, CH_2), 4.34—4.30(m, 1H, CH), 4.19—4.16(m, 1H, CH), 4.14—4.12(m, 1H, CH), 4.04—3.96(m, 2H, CH_2), 3.92(s, 3H, CH_3), 3.69—3.60(m, 11H, CH_3, CH_2), 3.56(s, 1H, CH), 3.38(t, *J*=6.0 Hz, 2H, CH_2), 3.24(s, 3H, CH_3), 2.85(s, 1H, CH), 2.63—2.58(m, 1H, CH), 2.56(s, 3H, CH_3), 2.16—2.12(m, 1H, CH), 2.04—1.67(m, 6H, CH_2), 1.60—1.36(m, 4H, CH_2), 0.98(s, 9H, CH_3), 0.76(t, *J*=7.4 Hz, 3H, CH_3). HRMS, *m*/*z*, calcd. for $C_{52}H_{70}N_{10}O_{10}S([M+H]^+)$: 1026.4997, found: 1026.4992.

(2*S*,4*R*)-1-[(*S*)-18-(Tert-butyl)-1-(4-{[(*S*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxyphenyl)-1,16-dioxo-5,8,11,14-tetraoxa-2,17-diazanonadecan-19-oyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide(**19**e): yield 45%, ^{1}H NMR(400 MHz, $CDCl_3$), *δ*: 8.67(s, 1H, thiazole-H), 7.94(d, *J*=8.1 Hz, 1H, ArH), 7.87(s, 1H, pyrimidine-H), 7.66—7.56(m, 2H, ArH), 7.48—7.34(m, 4H, ArH), 4.80—4.76(m, 1H, CH), 4.68—4.64(m, 1H, CH), 4.52—4.44(m, 2H, CH_2), 4.30—4.26(m, 1H, CH), 4.20—4.09(m, 4H, CH_2), 3.92(s, 3H, CH_3), 3.70—3.52(m, 15H, CH_3, CH_2), 3.36—3.30(m, 2H, CH_2), 3.24(s, 3H, CH_3), 2.54(s, 3H, CH_3), 2.42—2.38(m, 1H, CH), 2.14—2.10(m, 1H, CH), 2.04—1.68(m, 6H, CH_2), 1.54—1.36(m, 4H, CH_2), 0.98(s, 9H, CH_3), 0.76(t, *J*=7.4 Hz, 3H, CH_3). HRMS, *m*/*z*, calcd. for $C_{54}H_{74}N_{10}O_{11}S([M+H]^+)$: 1070.5259, found: 1070.5252.

(2*S*,4*R*)-1-[(*S*)-12-(Tert-butyl)-1-(4-{[(*S*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxyphenyl)-1,10-dioxo-5,8-dioxa-2,11-diazatridecan-13-oyl]-4-hydroxy-*N*-{(R)-1-[4-(4-methylthiazol-5-yl)phenyl]-ethyl}pyrrolidine-2-carboxamide(**19**f): yield 50%, ^{1}H NMR (400 MHz, $CDCl_3$), *δ*: 8.68(s, 1H, thiazole-H), 7.96(d, *J*=8.1 Hz, 1H, ArH), 7.86(s, 1H, pyrimidine-H), 7.64—7.58(m, 2H, ArH), 7.78—7.41(m, 4H, ArH), 4.95—4.90(m, 1H, CH), 4.56(t, *J*=8.4 Hz, 1H, CH), 4.50—4.44(m, 1H, CH), 4.18—4.12(m, 1H, CH), 4.05(s, 2H, CH_2), 3.94(s, 3H, CH_3), 3.90—3.88(m, 1H, CH), 3.80—3.60(m, 5H, CH_3, CH_2), 3.58—3.50(m, 4H, CH_2), 3.40—3.34(m, 2H, CH_2), 3.24(s, 3H, CH_3), 2.52(s, 3H, CH_3), 2.14—1.66(m, 8H, CH_2), 1.60—1.36(m, 7H, CH_2, CH), 0.98(s, 9H, CH_3), 0.76(t, *J*=7.4 Hz, 3H, CH_3). HRMS, *m*/*z*, calcd. for $C_{51}H_{68}N_{10}O_9S([M+H]^+)$: 996.4891, found: 996.4886.

(2*S*,4*R*)-1-[(*S*)-15-(Tert-butyl)-1-(4-{[(*S*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxyphenyl)-1,13-dioxo-5,8,11-trioxa-2,14-diazahexadecan-16-oyl]-4-hydroxy-*N*-{(*R*)-1-[4-(4-methylthiazol-5-yl)phenyl]ethyl}pyrrolidine-2-carboxamide (**19**g): yield 60%, ^{1}H NMR(400 MHz, $CDCl_3$), *δ*: 8.69(s, 1H, thiazole-H), 7.96(d, *J*=8.1 Hz, 1H, ArH), 7.87(s, 1H, pyrimidine-H), 7.68—7.56(m, 2H, ArH), 7.48—7.40(m, 4H, ArH), 4.94—4.89(m, 1H, CH), 4.56(t, *J*=8.4 Hz, 1H, CH), 4.49—4.42(m, 1H, CH), 4.19—4.12(m, 1H, CH), 4.10(s, 2H, CH_2), 3.92(s, 3H, CH_3), 3.90—3.86(m, 1H, CH), 3.79—3.56(m, 9H, CH_3, CH_2), 3.54—3.48(m, 4H, CH_2), 3.35—3.20(m, 5H, CH_2, CH_3), 2.50(s, 3H, CH_3), 2.16—1.66(m, 8H, CH_2), 1.60—1.36(m, 7H, CH, CH_2), 1.02(s, 9H, CH_3), 0.76(t, *J*=7.4 Hz, 3H, CH_3). HRMS, *m*/*z*, calcd. for $C_{53}H_{72}N_{10}O_{10}S([M+H]^+)$: 1040.5154, found: 1040.5156.

(2*S*,4*R*)-1-[(*S*)-18-(Tert-butyl)-1-(4-{[(*S*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxyphenyl)-1,16-dioxo-5,8,11,14-tetraoxa-2,17-diazanonadecan-19-oyl]-4-hydroxy-*N*-{(*R*)-1-[4-(4-methylthiazol-5-yl)phenyl]ethyl}pyrrolidine-2-carboxamide (**19**h): yield 60%, ^{1}H NMR(400 MHz, $CDCl_3$), *δ*: 8.64(s, 1H, thiazole-H), 7.92(d, *J*=8.1 Hz, 1H, ArH), 7.86(s, 1H, pyrimidine-H), 7.64—7.56(m, 2H, ArH), 7.47—7.40(m, 4H, ArH), 4.95—4.89(m, 1H, CH), 4.57(t, *J*=8.4 Hz, 1H, CH), 4.50—4.44(m, 1H, CH), 4.16—4.12(m, 1H, CH), 4.08(s, 2H, CH_2), 3.94(s, 3H, CH_3), 3.90—3.88(m, 1H, CH), 3.79—3.75(m, 1H, CH), 3.72—3.48(m, 15H, CH_3, CH_2), 3.54—3.48(m, 2H, CH_2), 3.36—3.30(m, 2H, CH_2), 3.24(s, 3H, CH_3), 2.52(s, 3H, CH_3), 2.16—1.66(m, 8H, CH_2), 1.60—1.36(m, 7H, CH_3, CH), 0.98(s, 9H, CH_3), 0.76(t, *J*=7.4 Hz, 3H, CH_3). HRMS, *m*/*z*, calcd. for $C_{55}H_{76}N_{10}O_{11}S([M+H]^+)$: 1084.5416, found: 1084.5411.

2.3 Biological Activity

2.3.1 Fluorescence Anisotropy Binding Assay[16]

The binding of compounds to BRD4 was assessed using a Fluorescence Anisotropy Binding Assay. All components were dissolved in a buffer composed of 50 mmol of HEPES, pH=7.4, 150 mmol of NaCl and 0.5 mmol of CHAPS with final concentrations of 40 nmol/L for BRD4 and 5 nmol/L for fluorescent ligand. To this reaction mixture were added various concentrations of test compound or DMSO vehicle(0.5% final) in corning 384 well Black low volume plates and equilibrated in dark for 4 h at room temperature. Fluorescence anisotropy was read on a Multi-Mode Microplate Reader(λ_{ex}=485 nm, λ_{em}=530 nm; $\lambda_{dichroic}$=505 nm).

2.3.2 CCK-8 Assay[6]

The human acute leukemia RS4;11 cells(purchased from the ATCC) were seeded in 96-well cell culture plates at a density of 1×10^4 cells/well in 100 μL of culture medium(RPMI 1640 media supplemented with 10% FBS and 1% penicillin streptomycin). Each compound tested was serially diluted in the appropriate medium, and 100 μL of the diluted solution

containing the tested compound was added to the appropriate wells of the cell plate. After the addition of the tested compound, the cells were incubated for 4 d at 37 °C in an atmosphere of 5% CO_2. The CCK-8 reagent was added to the plate, incubated for at least 1 h, and read at 450 nm. The readings were normalized to the DMSO-treated cells, and the IC_{50} was calculated by GraphPad Prism 6 software.

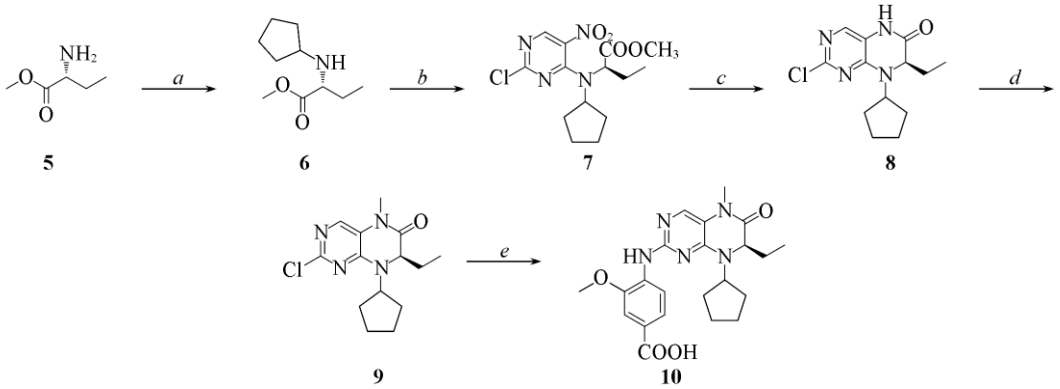
2.3.3 Western Blot Assay[6]

RS4;11 with a concentration of 2×10^6 cells/well was treated with compounds at the indicated concentrations for 6 h. Cells were collected and lysed in an RIPA buffer containing protease inhibitors. An amount of 20 μg of lysate was run in each lane of a PAGE-SDS and blotted into PVDF membranes. The antibodies for immunoblotting BRD4 were purchased from AmyJet Scientific Inc.(BioVision, USA) and GAPDH were purchased from AmyJet Scientific Inc.(Abbkine USA).

3 Results and Discussion

3.1 Chemistry

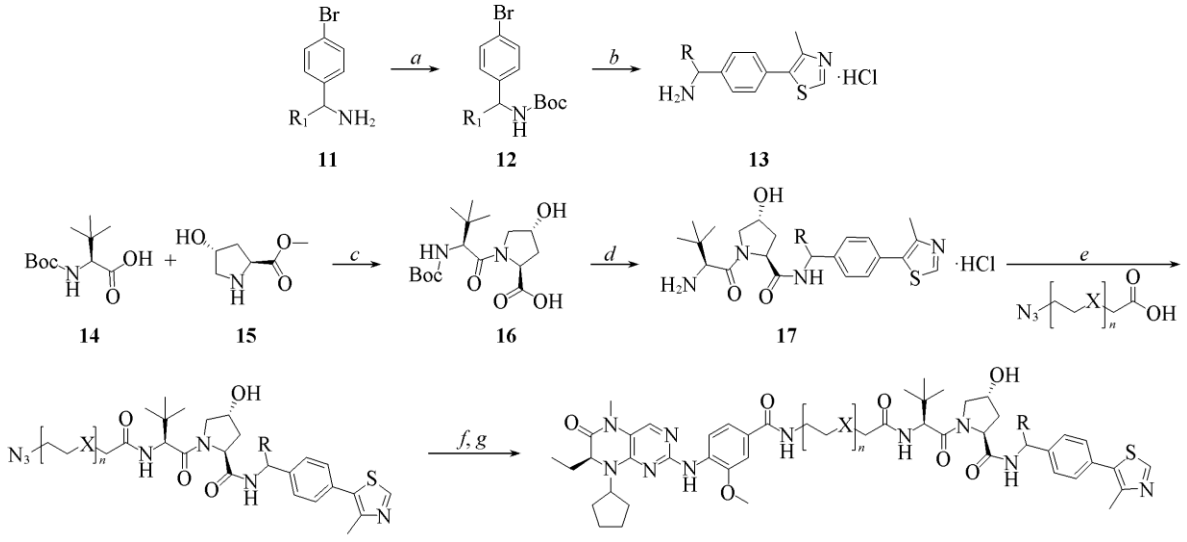
The synthesis of compound **10** is shown in Scheme 1[17]. (*R*)-Methyl 2-aminobutanoate(**5**) reductive amination with cyclopentanone afforded compound **6**. The secondary amine was reacted with 2,4-dichloro-5-nitropyrimidin to form compound **7**. Then the reduction of the nitro group was performed using iron and glacial acetic acid leading to an *in situ* intramolecular cyclization affording compound **8**. Methylation of the secondary amine and amination of the chloropyrimidine by 4-amino-3-methoxybenzoic acid afforded the desired compound **10**.



Scheme 1 Synthesis of compound 10

Reaction conditions: *a*. $NaBH(OAc)_3$, NaOAc; *b*. K_2CO_3, acetone; *c*. Fe, AcOH; *d*. NaH, DMF; *e*. EtOH, HCl.

The synthesis of compounds **19** is shown in Scheme 2[14]. Briefly, the amino of compound **11** was protected by boc-group to afford compound **12**. Compound **12** was coupling with 4-methylthiazole and deprotection to give compound **13.** Compounds **14** and **15** amidation and hydrolysis afforded compound **16**. Compound **16** amidated with azide carboxyl linker to obtain compound **18**. The azide group of compound **18** was reduced by Pd/C, and then amidated with compound **10** to give the target compound **19**.



18a: *n*=2, X=C, R=H
18b: *n*=2, X=C, R=(*S*)-CH_3
18c: *n*=2, X=O, R=H
18d: *n*=3, X=O, R=H
18e: *n*=4, X=O, R=H
18f: *n*=2, X=O, R=(*S*)-CH_3
18g: *n*=3, X=O, R=(*S*)-CH_3
18h: *n*=4, X=O, R=(*S*)-CH_3

19a: *n*=2, X=C, R=H
19b: *n*=2, X=C, R=(*S*)-CH_3
19c: *n*=2, X=O, R=H
19d: *n*=3, X=O, R=H
19e: *n*=4, X=O, R=H
19f: *n*=2, X=O, R=(*S*)-CH_3
19g: *n*=3, X=O, R=(*S*)-CH_3
19h: *n*=4, X=O, R=(*S*)-CH_3

Scheme 2 Synthesis of compounds 19

Reaction conditions: *a*. $(Boc)_2O$, $NaHCO_3$; *b*. (1) $Pd(OAc)_2$, KOAc; (2) 4 mol/L HCl in MeOH; *c*. (1) HATU, DIPEA, DMF; (2) LiOH, THF, H_2O; *d*. (1) HATU, DIPEA, DMF; 4 mol/L HCl in MeOH; *e*. HATU, DIPEA, DCM; *f*. Pd/C, MeOH; *g*. HATU, DIPEA, DCM.

3.2 Biological Activity of the Target Compounds

Accordingly, we designed and synthesized a series of potential BRD4 degrader using compound **10** for the BRD4 inhibitor portion, VHL ligand **1** or **2** as the VHL ligand and different length and composition linker. At the same time, their BRD4 inhibitory activity, RS4;11 cell growth inhibitory activity and BRD4 degrade activity were evaluated.

BRD4 inhibitory activities of the compounds were evaluated by the Fluorescence Anisotropy Binding Assay. All the compounds show BRD4 inhibitory activity(Table 1). First, compounds **19**a use C7 alkyl as the linker and VHL ligand **1** as the VHL ligand, the inhibitory activity of BRD4 is lower than that of BI2536. Next, we chose polyethylene glycol as the linker, and investigated the optimal linker length for BRD4 potencies, which resulted in compounds **19**c**—19**e. Compound **19c** with a diethylene glycol linker, which is as the same length as compound **19a,** shows better BRD4 inhibitory activity than compounds BI2536 and **19**a. Compound **19**d with a triethylene glycol linker is more potent than compound **19**c. However, compound **19**e with a tetraethylene glycol linker longer than that in compound **19**d is less potent than compound **19**d**.** Then, replacing the VHL ligand **1** to VHL ligand **2** resulted in compounds **19**b and compound **19**f**—19**h. All the compounds with the VHL ligand **2** show more potent BRD4 inhibitory activity than the compounds with the VHL ligand **1** having the same linker. In all the compounds, compound **19**g[IC_{50}=(18.6±1.3) nmol/L] shows better BRD4 inhibition activity than others.

Table 1 BRD4(BD1+BD2) inhibitory activity of the compounds

Compound	IC_{50}^{*}/(nmol L^{-1})
BI2536	94.6±8.7
19a	245.8±10.6
19b	208.2±16.5
19c	74.8±6.5
19d	42.6±2.5
19e	62.8±5.8
19f	50.6±3.2
19g	18.6±1.3
19h	46.4±4.6

* Mean values of deviation of triplicate experiments were given.

Then, we investigate the cancer cell growth inhibitory activity of all the compounds on RS4;11 cell lines(Table 2). All the compounds exhibit potent anti-proliferative activity**,** and the structure-activity relationship with the cell growth inhibitory activity is the same to the BRD4 inhibitory activity. Compound **19**g[IC_{50}=(34.2±4.3) nmol/L] shows more potent inhibitory growth activity for RS4;11 cell than other compounds.

Table 2 RS4;11 cell growth inhibitory activity of the compounds

Compound	IC_{50}^{*}/(nmol L^{-1})
BI2536	132.4±8.2
19a	335.3±10.2
19b	259±8.4
19c	102.6±7.6
19d	48.2±5.6
19e	63.7±4.5
19f	83.2±6.2
19g	34.2±4.3
19h	50.6±5.6

* Mean values of deviation of triplicate experiments were given.

We also examined the ability of compounds BI2536 and **19**g to induce BRD4 degradation in the RS4;11 cell lines, and the data are shown in Fig.2. The Western blotting analysis shows that compound **19**g, at concentrations of 0.4—0.6 μmol/L, is effective in decreasing the level of BRD4 protein in the RS4;11 cells, whereas the compound BI2536 at 0.8 μmol/L still fails to decrease the level of BRD4 protein. It can be concluded that compound **19**g is a potent BRD4 protein degrader.

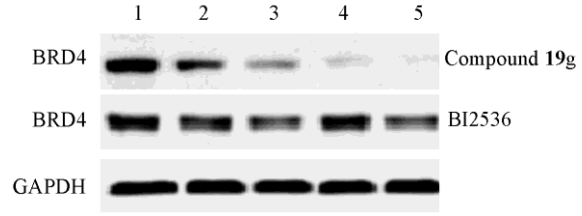


Fig.2 Western blotting analysis of BRD4 proteins in RS4;11 cells treated with compounds BI2536 and 19g

Lanes: 1. DMSO; 2. 0.2 μmol/L; 3. 0.4 μmol/L; 4. 0.6 μmol/L; 5. 0.8 μmol/L . RS4;11 cells were treated for 6 h with each individual compound at indicated concentrations and proteins were probed by specific antibodies. GAPDH was used as the loading control.

4 Conclusions

We designed a new class of PROTAC small-molecule degraders of BRD4 protein. Through extensive optimization of the VHL ligand and linker, we have obtained a number of highly potent small-molecule BRD4 protein degraders. One of the most promising compound **19**g exhibited robust potency of BRD4 inhibition with IC_{50} values of (18.6±1.3) nmol/L, respectively. Furthermore, compound **19**g potently inhibited cell proliferation in BRD4-sensitive cell lines RS4;11 with IC_{50} value of (34.2±4.3) nmol/L and capable of inducing degradation of BRD4 protein at 0.4—0.6 μmol/L in the RS4;11 leukemia cells. Collectively, our data demonstrate that compound **19**g is a highly potent, efficacious, and promising BRD4 degrader and warrants further evaluation as a potential new therapy for the treatment of human acute leukemia and other types of human cancer.

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